Neurobiology of Aging 34 (2013) 2379–2388

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Impact of ApoB-100 expression on cognition and brain pathology in wild-type and hAPPsl mice

Tina Löffler [a,b,*], Stefanie Flunkert [a], Daniel Havas [a], Miklós Sántha [c], Birgit Hutter-Paier [a], Ernst Steyrer [b], Manfred Windisch [a]

[a] QPS Austria, Grambach, Austria
[b] Institute of Molecular Biology and Biochemistry, Medical University Graz, Graz, Austria
[c] Institute of Biochemistry, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

ARTICLE INFO

Article history:
Received 7 January 2013
Received in revised form 12 March 2013
Accepted 3 April 2013
Available online 3 May 2013

Keywords:
Alzheimer's disease
Apolipoprotein B-100
Hyperlipidemia
Vascular risk factor
Transgenic mice
Brain

ABSTRACT

During their lifetime, people are commonly exposed to several vascular risk factors that may affect brain ageing and cognitive function. In the last few years, increasing evidence suggests that pathological plasma lipid profiles contribute to the pathogenesis of late-onset Alzheimer's disease. Importantly, hypercholesterolemia, especially elevated low-density lipoprotein cholesterol values, that is, increased apolipoprotein B-100 (ApoB-100) levels, represents an independent risk factor. In this study, the effects of ApoB-100 overexpression, either alone or in combination with cerebral expression of human amyloid precursor protein (hAPP), on cognitive functions and brain pathology were assessed. Our results show that ApoB-100 overexpression induces memory decline and increases cerebral lipid peroxidation and amyloid beta levels compared to those in wild-type animals. Although double-transgenic ApoBxAPP animals did not develop more distinct behavioral deficits than single-transgenic hAPP littermates, hApoB-100 expression caused additional pathophysiological features, such as high LDL and low HDL-cholesterol levels, increased lipid peroxidation, and pronounced ApoB-100 accumulation in cerebral vessels. Thus, our results indicate that ApoBxAPP mice might better reflect the situation of elderly humans than hAPPsl overexpression alone.
© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Alzheimer's disease (AD), a progressive neurodegenerative disease that is highly associated with accumulation and deposition of amyloid β (Abeta) peptides, is the most common form of dementia (Burns et al., 2002; Ferri et al., 2005). Generally, the disease can be separated into (1) heritable early-onset AD (EOAD) linked to mutations in the genes for Abeta Precursor Protein (APP) as well as presenilins (Tanzi and Bertram, 2001); and (2) so-called late-onset AD (LOAD) with unknown origin, which accounts for more than 95% of all AD cases (Harman, 2006). Especially because of the genes found to be affected in EOAD patients, the main research focus was drawn on the investigation of Abeta generation, processing, and clearance. Although the approved "amyloid cascade hypothesis" implies an imbalance between increased production and decreased clearance of Abeta as the triggering factor in AD (Hardy and Selkoe, 2002), the underlying causes for the shifted balance in LOAD remain unclear.

In the last years, increasing evidence suggests that hypercholesterolemia and other vascular factors may contribute to the pathogenesis of LOAD (Humpel, 2011). Notably, AD, atherosclerosis, and especially coronary artery disease (CAD) share many risk factors, such as diabetes, hypertension, hyperlipidemia, and the inheritance of an apolipoprotein E4 (ApoE4) allele (Corder et al., 1993; Jarvik et al., 1995; Skoog et al., 1999). In addition to the ApoE genotype, apolipoprotein B (ApoB) is also associated with both CAD and AD (Kuo et al., 1998; Martins et al., 2009). ApoB is known as the primary apolipoprotein of cholesterol-carrying, low-density lipoproteins (LDL) and triglyceride-rich, very-low-density lipoproteins (VLDL). CAD and AD patients share a very similar pathological plasma lipid profile, exhibiting increased levels of LDL along with decreased high-density lipoprotein (HDL) levels (Kuo et al., 1998; Sparks et al., 2000). Intriguingly, a post-mortem study of AD patients showed that LDL and ApoB levels positively correlate with brain Abeta42 levels (Kuo et al., 1998). In addition, several cerebrovascular abnormalities have been identified in AD brains: decreased microvascular density, an impaired blood–brain barrier, and cerebral amyloid angiopathy (CAA) (Farkas and Luiten, 2001). So far it is still under debate as to what extent these pathologies contribute to the development of LOAD and how they influence the time course of progression. In this study, the effects of ApoB-100

* Corresponding author at: Institute of Molecular Biology and Biochemistry, Medical University Graz, Harrachgasse 21, 8010 Graz, Austria. Tel.: +43 316 258 111 215; fax: +43 316 258 111 300.
E-mail address: Tina.Loeffler@qps.com (T. Löffler).

0197-4580/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2013.04.008

expression, either alone or in combination with cerebral expression of human amyloid precursor protein (hAPP), on cognitive functions and brain pathology were assessed.

ApoB-100 animals, overexpressing the entire 43-kb human ApoB-100 gene including its natural human promoter (Bjelik et al., 2006; Callow et al., 1994) were originally designed as a model of hyperlipidemia and atherosclerosis. Because the murine plasma lipid profile has a very low LDL to HDL ratio compared to the human profile, wild-type mice are protected against hypercholesterolemia and are usually not affected by atherosclerosis (Breslow, 1996). It has been shown that overexpression of human ApoB-100 in mice shifted the lipoprotein profile to a more atherogenic phenotype. Accordingly, atherosclerotic lesions were detected at branch points of the aorta, especially as a consequence of a high-cholesterol diet (Callow et al., 1995). Recent publications showed that ApoB-100–overexpressing animals display cerebral microvascular lesions, changes in APP metabolism, and some plaque-like accumulations in their brains (Bereczki et al., 2008; Bjelik et al., 2006; Sule et al., 2009). In contrast, the hAPPsl transgenic mouse line is a well-described model of familial AD. These animals are highly overexpressing human APP751 with London (V717I) and Swedish (KM670671NL) mutations under the control of the murine Thy-1 promoter, as first described by Rockenstein et al. (2001). The hAPPsl mice display severe brain amyloid pathology associated with early-onset progressive memory deficits (Havas et al., 2011).

The aim of the present study was to crossbreed hAPPsl with ApoB-100 mice to introduce a vascular risk factor into the existing AD mouse model to better mimic the situation in elderly people and to investigate the interplay between the 2 pathologies. Effects of ApoB-100 overexpression, but also possible synergisms in ApoBxAPP animals, on several biochemical and histological markers as well as the behavioral phenotype, were analyzed in an age-dependent manner.

2. Methods

2.1. Animals

By heterozygous crossbreeding of ApoB-100 transgenic (Bjelik et al., 2006) and hAPPsl mice (Rockenstein et al., 2001), both on a C57Bl/6J background, 4 different genotypes were obtained: double transgenic ApoBxAPP, single-transgenic ApoB-100, single-transgenic hAPPsl, and nontransgenic (wild-type [WT]) littermates. From every genotype, 10 male and 10 female mice were tested for behavioral deficits at 4, 6, 9, and 12 months of age.

Animals were housed in individually ventilated cages under a constant light–dark cycle (12 hours light/dark). Room temperature and humidity were kept constant at approximately 24 °C and 40% to 70%, respectively. Dried, pelleted standard rodent chow (Altromin, Lage, Germany) and normal tap water were available ad libitum.

Animal studies conformed to the Austrian guidelines for the care and use of laboratory animals and were approved by the Styrian Government, Austria.

2.2. Tissue sampling

From every tested mouse, blood and brain tissue were sampled after sedation by standard inhalation anaesthesia. Blood was collected into heparin-coated vials and used to obtain plasma. After blood sampling, mice were transcardially perfused with physiological (0.9%) saline. Thereafter, brains were removed and hemisected. From the left hemisphere, the cortex and hippocampus were dissected and immediately frozen on dry ice and stored at −80 °C until biochemical examination. The right hemispheres of all mice were fixed by immersion in fresh 4% paraformaldehyde/phosphate-buffered saline, pH 7.4, at room temperature for 1 hour, followed by 24-hour incubation in 15% sucrose solution for cryo-conservation. Hemispheres were stored at −80 °C until histological processing.

2.3. Homogenization of frozen brain samples

The frozen tissue samples were weighed, and Tissue Homogenization Buffer (THB; 250 mmol/L sucrose, 1 mmol/L ethylenediaminetetraacetic acid (EDTA), 1 mmol/L ethylene glycol tetraacetic acid (EGTA), 20 mmol/L Tris, pH 7.4) including 1x protease inhibitor (Calbiochem, Darmstadt, Germany) was added. For cortex samples, 1 mL THB per 100 mg tissue was added; for hippocampal samples, 2.5 mL was used for 100 mg tissue. The tissue was homogenized with the Tissue Ruptor (Qiagen, Düsseldorf, Germany) for 20 seconds using the highest level.

2.4. Extraction of non–plaque-associated proteins (DEA-Fraction)

For extraction of non–plaque-associated proteins, 100 μL of the THB homogenate was mixed with 100 μL diethylamine (DEA) solution (0.4% DEA, 100 mmol/L NaCl). The mixture was centrifuged for 1 hour at 74,200 × *g*, 4 °C. A 170-μL quantity of the supernatant was transferred to a 1.5-mL Eppendorf tube and neutralized with 17 μL 0.5 mol/L Tris, pH 6.8.

2.5. Extraction of deposited proteins (FA-Fraction)

For extraction of deposited proteins, 100 μL of the THB homogenate was mixed with 220 μL cold formic acid (FA) and sonificated for 1 minute on ice. A 300-μL quantity of this solution was transferred to a centrifugation tube and centrifuged for 1 hour at 74,200 × *g*, 4 °C. After centrifugation, 210 μL of the supernatant was transferred to a fresh tube and mixed with 4 mL FA Neutralization Solution (1 mol/L Tris, 0.5 mol/L Na_2HPO_4, 0.05% NaN_3).

2.6. Lipid peroxidation measurements

A 50-μL quantity of the THB homogenates was mixed with 50 μL 5% SDS solution including 1x protease inhibitor as well as 1x butylhydroxytoluene (BHT) solution (5 mmol/L) and sonificated for 5 seconds. Malondialdehyde (MDA) was used as a standard at final concentrations between 1 and 20 μmol/L in 1:1 (v/v) mixtures of THB and 5% SDS. The thiobarbituric acid reactive species assay (TBARS-assay) was started by adding 55 μL of 1.33% TBA and 95 μL of 20% acetic acid, pH 3.5, to 100 μL of the prepared samples or standards. After 1 hour incubation at 95 °C, 250 μL of n-Butanol/Pyridin (15:1 v/v) was added, and the solutions were mixed by gently inverting the tubes. After centrifugation for 10 minutes at 4000 × *g*, 200 μL of the upper organic phase was transferred to 1 well of a 96-well plate, and absorbance was measured at 535 nm with a μQuant plate photometer.

2.7. Plasma lipid measurements

For the determination of total cholesterol, triglycerides and HDL-cholesterol Fluitest CHOL, Fluitest TG, and Fluitest HDL direct (Analyticon Biotechnologies, Lichtenfels, Germany) assays, respectively were used. Plasma of all animals was diluted 1:2 with 0.2% NaCl solution and the assays were carried out according to the supplied manuals. LDL cholesterol content was measured with Fluitest LDL direct (Analyticon Biotechnologies, Lichtenfels, Germany) assay, as well as calculated with Friedewald equation (LDL-chol = total chol − HDL chol − ([TG/5])).

Download English Version:

https://daneshyari.com/en/article/6806959

Download Persian Version:

https://daneshyari.com/article/6806959

Daneshyari.com